

WHAT IS CLAIMED IS:

1. A method of producing seeds of a monocot plant that accumulate a heterologous protein, which method comprises the following steps:

(a) stably transforming a plant cell of the monocot plant with a chimeric gene to obtain a transformed monocot plant cell, the chimeric gene comprising

(i) a promoter from a monocot non seed-storage protein gene,

(ii) a first DNA sequence, operably linked to said promoter, encoding a monocot seed-specific signal peptide capable of targeting a linked polypeptide to an intracellular region within a seed cell, and

(iii) a second DNA sequence, operably linked to said promoter and linked in translation frame with the first DNA sequence, encoding the heterologous protein, wherein the first DNA sequence and the second DNA sequence together encode a fusion protein comprising the signal peptide and heterologous protein;

(b) growing a plant from the transformed plant cell to produce seeds that express the heterologous protein; and

(c) harvesting the seeds from the plant grown in step (b) to obtain the seeds that accumulate the heterologous protein.

2. The method of claim 1, wherein the monocot seed-specific signal peptide is a monocot seed-specific N-terminal signal peptide.

3. The method of claim 1, wherein the monocot plant is a rice plant.

4. The method of claim 1, wherein the intracellular region is a protein body I, protein body II, starch granule, chloroplast, mitochondria or endoplasmic reticulum.

5. The method of claim 1, wherein the heterologous protein is an animal protein.

6. The method of claim 5, wherein the animal protein is a mammalian protein.
7. The method of claim 6, wherein the mammalian protein is a human protein.
8. The method of claim 7, wherein the human protein is selected from the group consisting of a blood protein, milk protein, human gastrointestinal peptide, lipase, amylase, colony stimulating factor, cytokine, interleukin, integrin, T cell receptor, immunoglobulin, growth factor and growth hormone of human origin.
9. The method of claim 8, wherein the human protein is selected from the group consisting of lysozyme, lactoferrin, lactoperoxidase, kappa-casein, hemoglobin, alpha-1-antitrypsin, fibrinogen, antithrombin III, serum albumin, trypsinogen, aprotinin, transferrin, growth hormone, antibody, insulin, insulin-like growth factor, epithelial growth factor, intestinal trefoil factor, granulocyte colony-stimulating factor and macrophage colony-stimulating factor of human origin.
10. The method of claim 1, wherein the promoter is a promoter of a gene selected from the group consisting of wheat purindoline b protein gene, protein disulfide isomerase gene and heat shock 70 protein gene.
11. A method of producing a substantially purified protein heterologous to a monocot plant, comprising the method of claim 1, and further comprising processing the seeds to obtain a fraction enriched for the heterologous protein, and purifying the heterologous protein from the enriched fraction to obtain the protein heterologous to the monocot plant.
12. A method of producing seeds of a monocot that accumulate a heterologous protein in at least two intracellular regions within a cell of the seeds of the monocot, which method comprises the steps of:

(a) stably co-transforming a cell of the monocot with at least first and second chimeric genes to obtain a transformed monocot cell, the first chimeric gene comprising

- (i) a first promoter from a monocot protein gene,
- (ii) a first DNA sequence, operably linked to the promoter, encoding a first monocot seed-specific signal peptide capable of targeting a polypeptide linked thereto to a first intracellular region within a monocot seed cell, and
- (iii) a second DNA sequence, operably linked to the first promoter and linked in translation frame with the first DNA sequence, encoding the heterologous protein, wherein the first and second DNA sequences together encode a fusion protein comprising the first monocot seed-specific signal peptide and the heterologous protein,

the second chimeric gene comprising

- (i) a second promoter from a monocot protein gene,
- (ii) a third DNA sequence, operably linked to the promoter, encoding a second monocot seed-specific signal peptide capable of targeting a polypeptide linked thereto to a second intracellular region within a monocot seed cell, and
- (iii) a fourth DNA sequence, operably linked to the second promoter and linked in translation frame with the third DNA sequence, encoding the heterologous protein, wherein the third and fourth DNA sequences together encode a fusion protein comprising the second monocot seed-specific signal peptide and the heterologous protein,

wherein the first and second promoter are different, the first and second monocot seed-specific signal peptides are different, and the first and second intracellular regions are different;

(b) growing a monocot plant from the transformed monocot cell to produce seeds that express the heterologous protein in at least two different intracellular regions; and

(c) harvesting the seeds from the monocot plant grown in step (b) to obtain the seeds of the monocot that accumulate the heterologous protein.

13. A method of producing seeds of a monocot that accumulate a heterologous protein in at least two different intracellular regions within a cell of the seeds of the monocot, which method comprises the steps of:

(d) stably transforming a first cell of the monocot with a first chimeric gene to produce a first transformed cell of the monocot, the first chimeric gene comprising

- (i) a first promoter from a monocot protein gene,
- (ii) a first DNA sequence, operably linked to the first promoter, encoding a first monocot seed-specific signal peptide capable of targeting a polypeptide linked thereto to a first intracellular region within a monocot seed cell, and
- (iii) a second DNA sequence, operably linked to the first promoter and linked in translation frame with the first DNA sequence of (a)(ii), encoding the heterologous protein, wherein the first and second DNA sequences together encode a fusion protein comprising the first monocot seed-specific signal peptide and the heterologous protein;

(e) stably transforming a second cell of the monocot with a second chimeric gene to produce a transformed second cell of the monocot, the second chimeric gene comprising

- (i) a second promoter from a monocot protein gene,
- (ii) a third DNA sequence, operably linked to the second promoter, encoding a second monocot seed-specific signal peptide capable of targeting a polypeptide linked thereto to a second intracellular region within a monocot seed cell, and
- (iii) a fourth DNA sequence, operably linked to the second promoter and linked in translation frame with the third DNA sequence of (b)(ii), encoding the heterologous protein, wherein the third and fourth DNA sequences together

encode a fusion protein comprising the second monocot seed-specific signal peptide and the heterologous protein, wherein the first and second promoter may be the same or different, the first and second monocot seed-specific signal peptides are different, and the first and second intracellular regions are different;

(f) growing a monocot plant from the first transformed cell of (a) to produce a first monocot plant that can express the heterologous protein in the first intracellular region;

(d) growing a monocot plant from the second transformed cell of (b) to produce a second monocot plant that can express the heterologous protein in the second intracellular region;

(e) crossing the first and second monocot plants to produce a hybrid plant;

(f) growing the hybrid plant to produce seeds that can express the heterologous protein in the first and second intracellular regions in the same seed cell; and

(g) harvesting the seeds from the hybrid plant to obtain the seeds of the monocot that accumulate the heterologous protein.